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THE EFFECTS OF CERTAIN GENES ON THE OUTER PISTILLATE GLUME OF MAIZE

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A comparison of the effects which various genes have on the structure of a given plant-organ should sometimes reveal which of the loci concerned were involved in the previous evolution of that organ and which of them might, during domestication of the species, contribute to its further evolution. The extreme susceptibility of the outer pistillate glume¹ in the maize tribe (*Maydeae*) and the related tribe *Andropogoneae* to evolutionary modifications suggests that the genetic variation affecting development of this organ in maize (*Zea Mays* L) may reflect some of its evolutionary changes. The changes in glume structure were drastic during evolution leading to the formation of the cupulate fruit case (Galinat, 1956). This organ evolved from a long foliaceous bract (*Erianthus* spp.) to a shorter, coriaceous structure marked by various types of sculpturing (*Manisuris* spp. ; *Hackelochloa* spp.) and, finally, to a highly lignified glume which is specialized in shape, texture and plane of divergence so as to bring about a closure of the narrow opening of a cupulate rachis-segment (*Euchlaena* and *Tripsacum*).

¹ Further mention of the glumes of maize will refer only to the outer pistillate ones.

Thus, we might expect that a study of the various glume phenotypes of modern maize would be revealing as to the past and possible further evolution of this glume during domestication.

Three dominant or semi-dominant genes which affect principally the outer pistillate glume were included in this study. They are as follows: (1) Papyrescent (*Pn* gene on chromosome-7); (2) Tunicate (*Tu* gene on chromosome-4); (3) Vestigial glume (*Vg* gene on chromosome-1). These genes were studied in heterozygous condition after they had been incorporated, by repeated backcrossing, into an isogenic background of a sweet corn (maize) inbred (Purdue 39). Their effects on the histological structure of this glume were examined in material prepared in the following manner: Normal (typical) and variant specimens were fixed at 18 days after pollination. This material was later embedded in paraffin, according to the usual procedure, and then cut in cross-section at 12 μ . The sections were then stained by the standard safranin-fast-green technique. Finally, a projection apparatus was used in making tracings from comparable slides.

Normal glumes. The glumes and other floral bracts of modern maize are so reduced that the mature grain emerges naked above them. Protection for the grain, which is provided by long floral bracts in most other grasses, is supplied by husks (modified leaf-sheaths) borne below the pistillate inflorescence (ear) on a condensed branch (shank).

The normal glume has papery lateral wings (Plate IX, fig. 3) and the entire structure is homologous to a leaf-sheath with sheath-auricles. The glume-sheath, which is the counterpart of the leaf-sheath, may be coriaceous in texture (in many South American varieties) or, more commonly, it may resemble its counterpart in teosinte

in being corneous or indurate (in teosinte-contaminated varieties from North and Central America). The degree of this induration has been used as an estimate of teosinte-introgression in archaeological maize, after the reliability of the method has been established (Galinat *et al*, 1956).

Internally, the mesophyll of the glume is divided into definite specialized regions. The induration is confined to a region of smaller cells extending from the outer epidermis to a line delimited by a row of vascular bundles about midway through a cross section (Plate X, fig. 3). As indicated by strong safranin staining, these smaller indurated cells are characterized by an accumulation of lignin in the secondary walls. The remainder of the glume, extending to the inner epidermis, consists of large parenchyma-cells. During final maturation of the glume, this parenchymatous tissue collapses from desiccation. The resulting shrinkage of the parenchyma toward the bundles causes an outline of the vascular system to be revealed as parallel ridges along the inner epidermis.

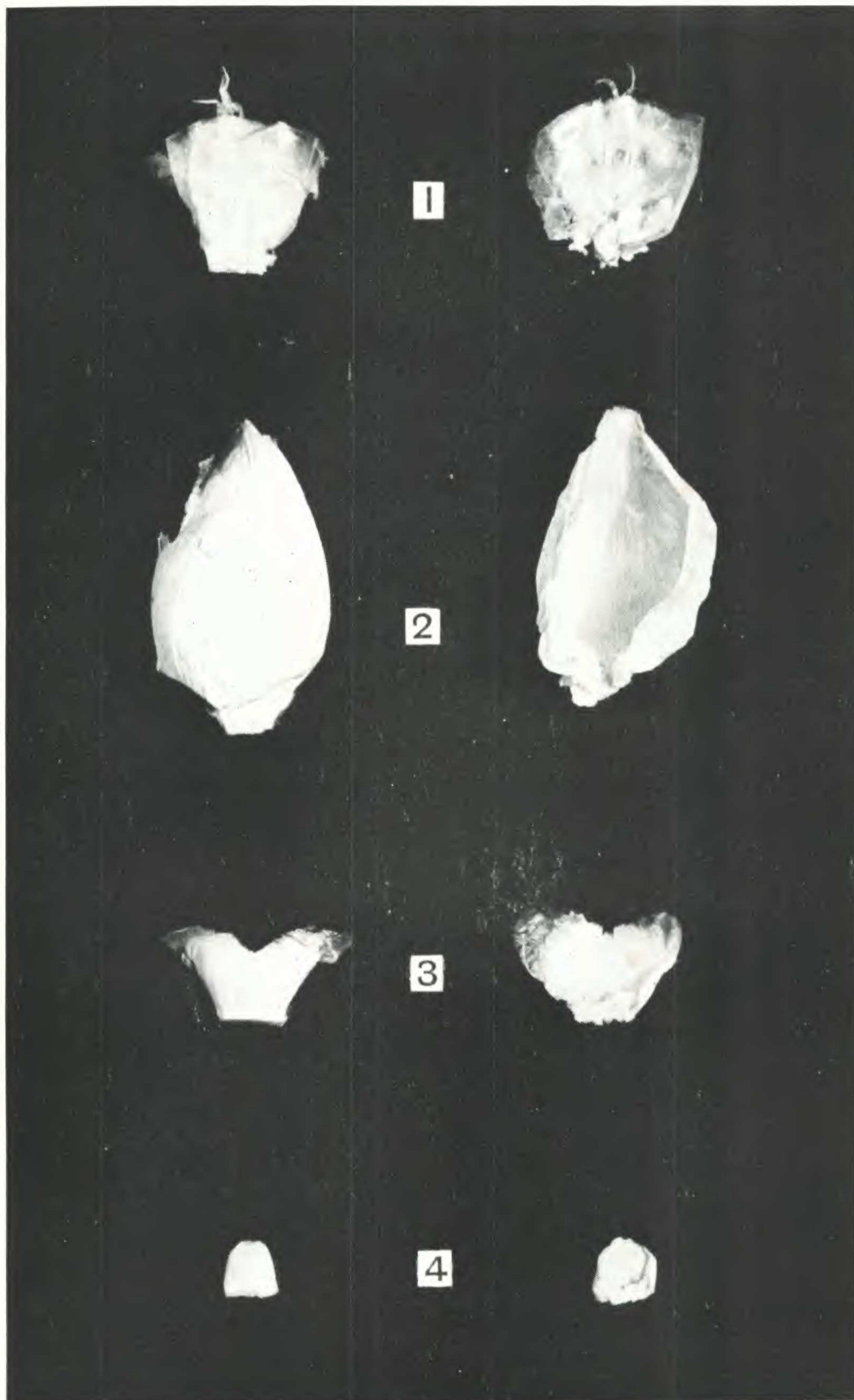
The vascular arrangement of the normal glume differs slightly from that which occurs in a vegetative leaf-sheath. The glume has smaller bundles and, since this organ is determinative, they converge at the apex. In the leaf-sheath these principal bundles remain parallel as they continue on into the blade. As in the leaf-sheath, the parallel bundles are of two sizes with the larger ones alternating with one or two smaller ones. The small cross connections which anastomose between the parallel bundles are confined to the apical region of the glume as compared to a distribution along the entire length of both the blade and sheath of the leaf. The glume wings are non-vascular as are the ligules and sheath-auricles of the leaf-sheath. It seems that these vascular differences between glumes and leaf-sheaths are more in the nature of minor modifications.

EXPLANATION OF THE ILLUSTRATION

PLATE IX. The outer pistillate glume of maize, as effected by various genes. Left and right specimens show dorsal and ventral views. 1, papyrescent (*Pn*); 2, tunicate (*Tu*); 3, normal; 4, vestigial glume (*Vg*). Twice natural size.

Photograph by FRANK WHITE

PLATE IX

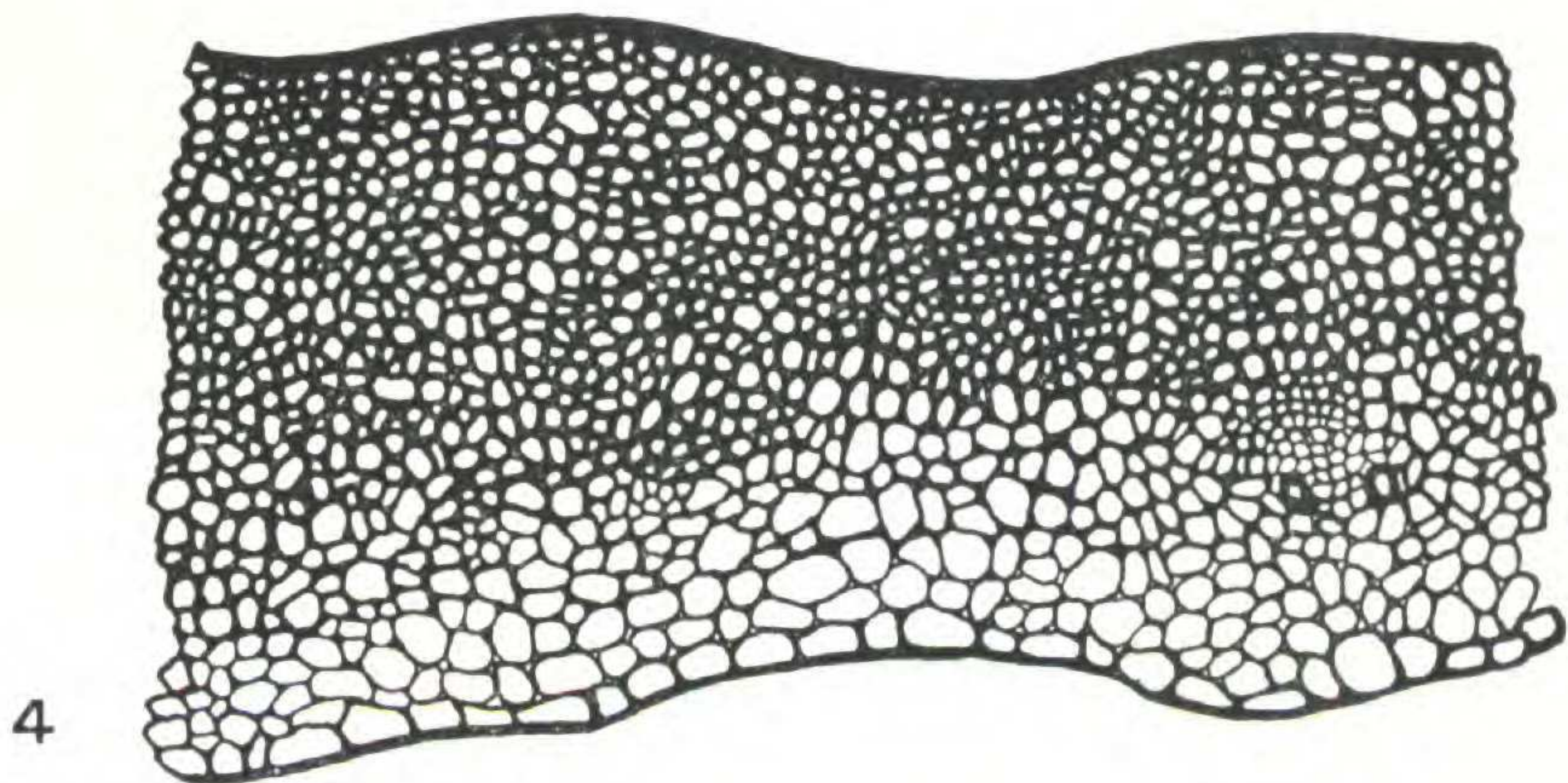
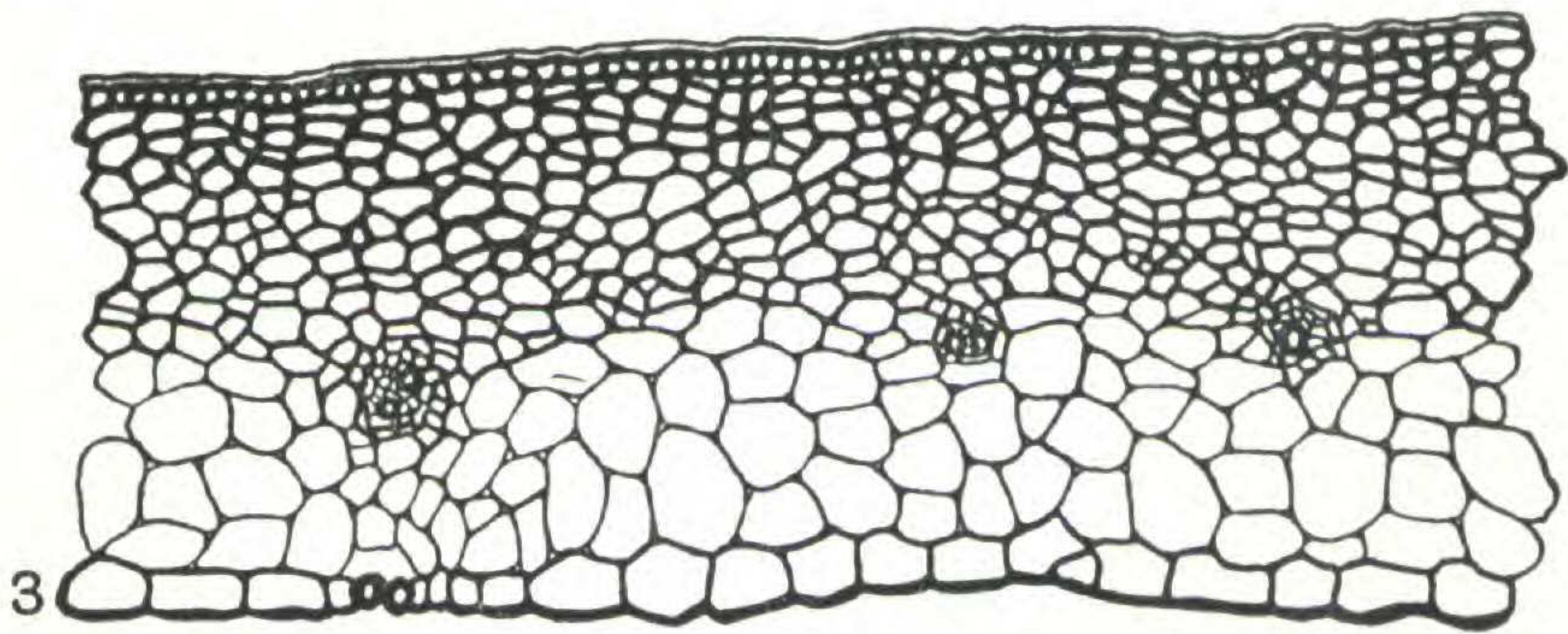
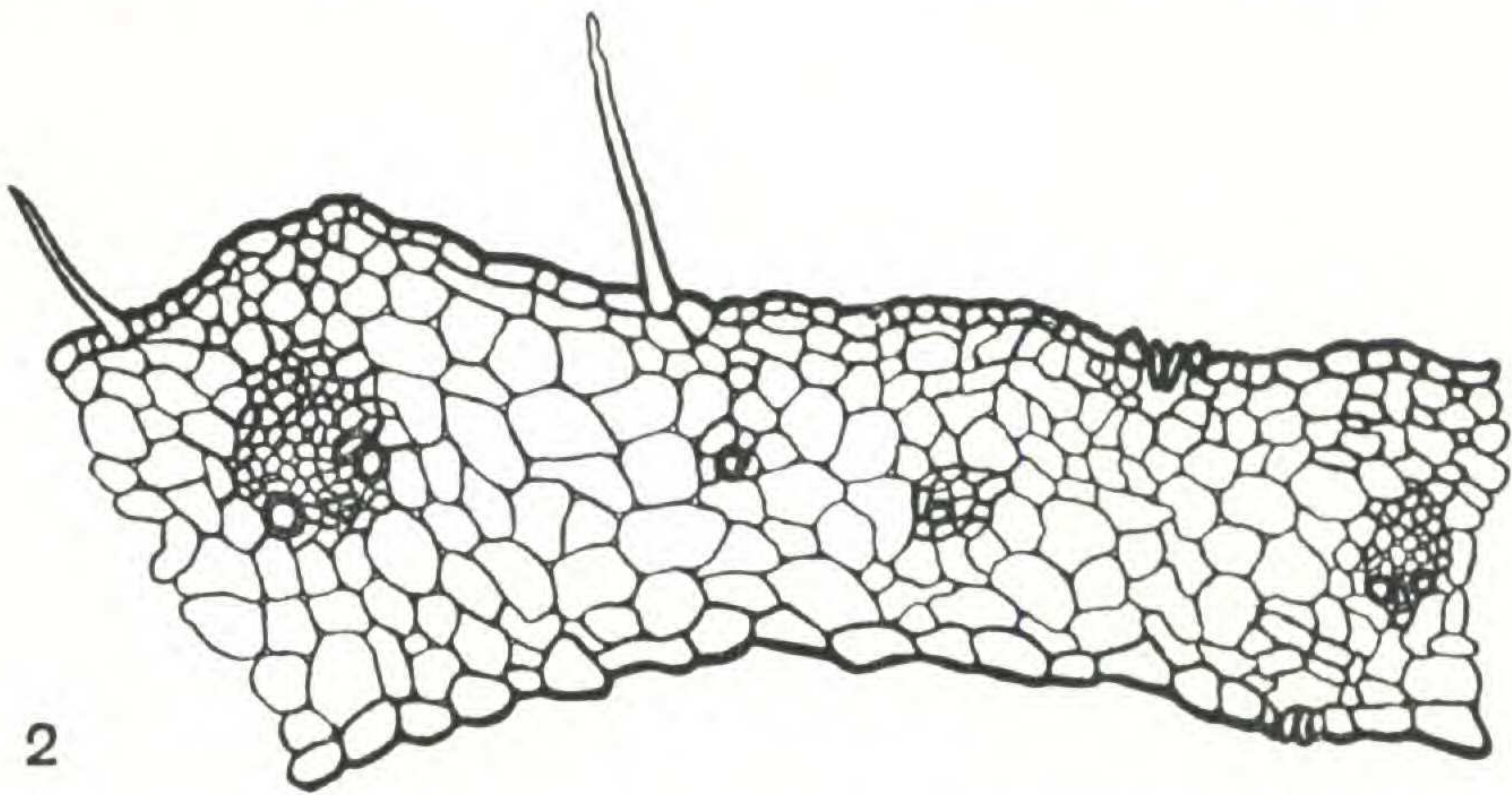
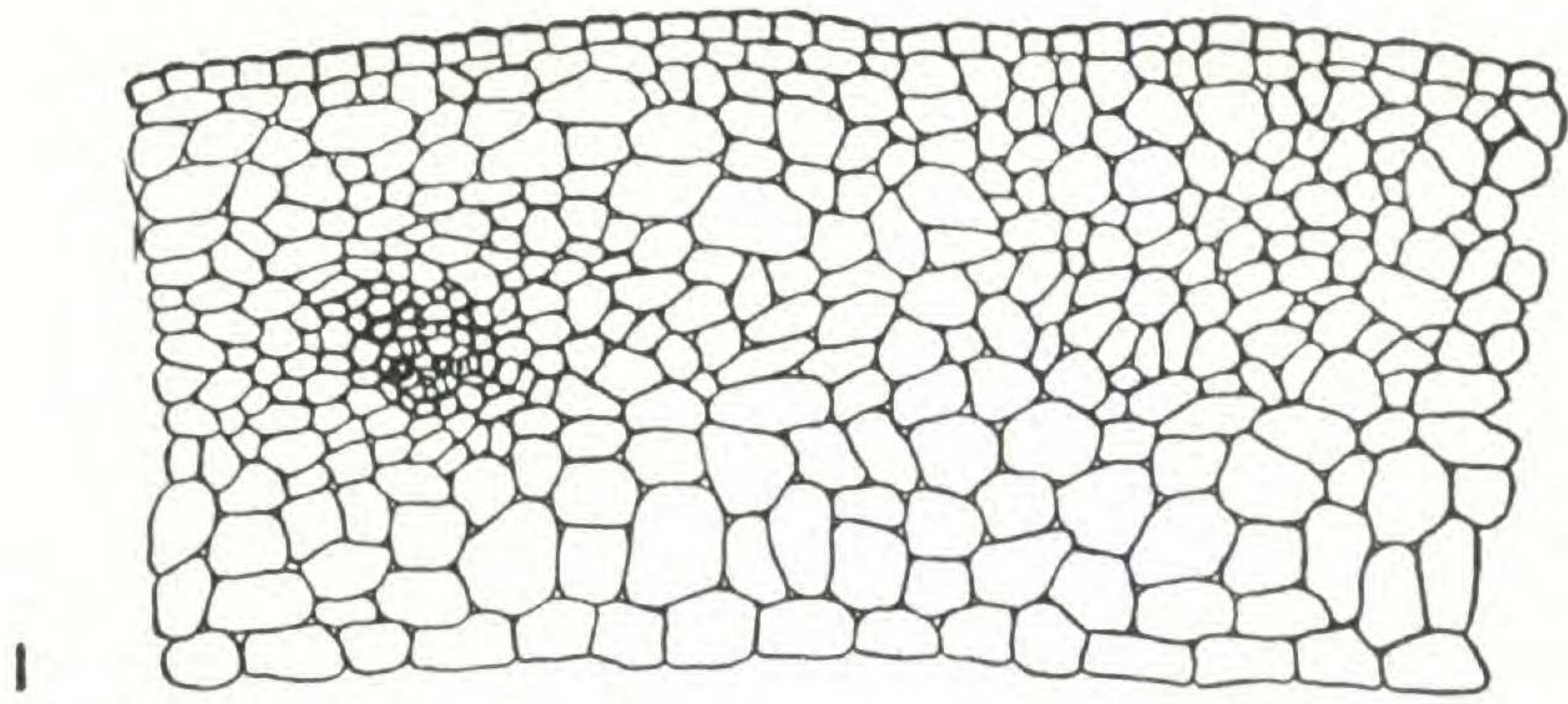


EXPLANATION OF THE ILLUSTRATION

PLATE X. Projection tracings of comparable cross-sectional views of the outer pistillate glume as this organ is affected by certain genes. In each figure the outer (abaxial) epidermis is uppermost. 1, papyrescent (*Pn*); 2, tunicate (*Tu*); 3, normal; 4, vestigial glume (*Vg*). About 80 times natural size.

Drawn by WALTON C. GALINAT

PLATE X



As in the leaf, stomata occur along both epidermal surfaces. In the glume, however, they are vestigial and obscure along the outer epidermis. Such reduction may result from the extreme lignification of the underlying tissue. Rather well-developed stomata are found on the inner surface directly opposite the larger vascular bundles. The fact that the maize-glume has stomata and yet does not have an opportunity to function in photosynthesis, seems to emphasize the leaf-like nature of this organ.

The outer epidermis has a glabrous cuticle and may acquire a brown, red or purple coloration from the action of certain genes. Epidermal pubescence is greatly reduced, except in the lateral wings.

Papyrescent glumes. This dominant mutant is characterized by glumes which upon final maturation become papery and similar in texture to that which occurs in lemmas, paleas and lateral wings of normal glumes, as well as in the ligules of normal leaves (Plate IX, fig. 1). These papyrescent glumes are usually slightly longer than the mature grain. A condition which is superficially similar to the papyrescent character develops in normal ears which are poorly matured. This simulation results from the protrusion of the lemmas, paleas and papery wings from normal glumes above chaffy or small grains.

The papyrescent character is likewise similar or identical with the "palee sviluppate" of Bonvicini (1932) and apparently with the recessive "semivestidos" of Andrés (1950). Our character for papyrescent glumes may have been incorrectly identified by Andrés as a recessive factor since the effects of this semi-dominant gene are not always readily apparent in the heterozygote. It has also been confused by Weatherwax (1954) with half-tunicate, from which, as will be pointed out later, it is histologically distinct. Furthermore, we find a counterpart of the

papyrescent condition in *Sorghum*, where it once led to the describing of a new species (*Sorghum papyrascens* Stapf), an error later corrected by Rangaswami and Panduranga (1936) who correctly identified it as a single gene mutant characterized by defective glume development. Papery glumes have also appeared in two X-ray induced mutants (*seminudoides* and *subnudoides*) of barley (Scholz, 1956). In these two recessive mutants, the floral bracts fragmentize away from the grain during threshing. Ordinarily the floral bracts of barley remain about the grain following threshing, except in the mutant named *nudoides*. The differences in the terminology of Scholz and Andrés, as applied to their particular mutants, refer to differences in the normal condition of the glumes in barley and maize. The term "seminudoides" (half-nude) applies to the barley mutant because normally the threshed barley grain is completely covered, while "semi-vestidos" (half-clothed) is better for the maize mutant because normally the maize grain is exposed.

Papyrescent glumes in maize consist largely of non-specialized parenchyma-cells which, at 18 days from pollination, are large and fleshy, being swollen with water (Plate X, fig. 1). During final maturation of the ear, dessication causes these parenchymatous glumes to shrink to a thin, almost transparent condition with the vascular bundles becoming prominent ridges (Plate IX, fig. 1). There is little contraction in length and width, however, and the glumes remain partially covering the surface of the mature grain. Finally, they become papery and brittle and are distinctly different from those glumes which protect the caryopses of other grasses.

The epidermal layers of the papyrescent glume approach those of the normal glume in regard to cell size, but differ from normal in being less lignified and in lacking stomata. Pubescence is usually confined to the mar-

ginal part of the glume. The vascular bundles are essentially normal in structure, although they are not so closely spaced.

The succulent nature of immature papyrescent glumes often encourages the destructive activities of ear-rotting fungi. This suggests that a hardening of these glumes, such as is caused by teosinte introgression, may promote resistance to pathogenic fungi.

The gene which produces this defective character has been previously designated as pseudopod (*Pp*) (Galinat and Mangelsdorf, 1955), but it now seems more desirable to change its name and symbol to papyrescent (*Pn*) (Galinat and Mangelsdorf, 1957). This change will call attention to its final papery character and its similarity to the "papyrascens" character of *Sorghum*, and will avoid confusion with the symbols for heterozygous pericarp color (*Pp*) as well. Our *Pn* gene was originally isolated from a Peruvian variety of maize (Mangelsdorf, 1948). It is located close to the *bd* (branched-silkless) gene near the end of the long arm of chromosome-7. Its linkage relations will be discussed in more detail in a future paper.

Tunicate glumes. The highest tunicate allele (*Tu*) from the series of multiple-alleles at the *Tu-tu* locus was chosen in order to study, in accentuated form, the effects of genes at this locus. Weaker alleles at the tunicate locus have intermediate effects on the length, shape and texture of the glume (Mangelsdorf, 1948). Further discussion of tunicate glumes will refer to the phenotype of the strongest tunicate allele (Plate IX, fig. 2).

The glumes of tunicate maize are like those of most other grasses in being long enough to enclose the grain and in being foliaceous. They differ therefore, in length and texture from the pistillate glumes characteristic of the American *Maydeae*, including normal maize, and of

Manisuris spp. in the *Andropogoneae*. In the evolutionary sequence leading to formation of the cupulate fruit case, tunicate glumes of maize are similar to the glumes of *Elyonurus tripsacoides* (Galinat, 1956).

Anatomically, tunicate glumes have all of the characteristics of a typical leaf-sheath, but on a reduced scale (Plate X, fig. 2). The close similarities of these organs may be enumerated as follows: (1) The inner (adaxial) and outer (abaxial) surfaces of tunicate glumes and leaf-sheaths are about equally pubescent with soft trichomes. This condition differs from that of the leaf-blade where pubescence is located chiefly on the upper (adaxial) epidermis and from that of the normal glume where the trichomes are confined to the lateral wings. (2) Both tunicate glumes and leaves (including sheath and blade) are herbaceous in texture because their mesophyll lacks the region of lignified cells which characterizes the normal glume. (3) Stomatal development in tunicate glumes resembles that of the leaf-sheath and differs from the condition found in the normal glume. Tunicate glumes and leaf-sheaths have well-developed stomata on both surfaces although they are more frequent and functional on the outer epidermis. As noted previously, if well-developed stomata occur in the normal glume, they are located on the inner surface. (4) The vascular system of tunicate glumes is more similar to the venation of leaves than it is to that of normal glumes. In both tunicate glumes and leaves, the anastomosing venation connects the principle bundles at irregular intervals along their entire length rather than just in the distal region as in the normal glume. (5) The structure of tunicate bundles is frequently identical to that of leaf bundles in contrast to the more reduced veins of normal glumes (Plate X, figs. 2 and 3). All of these anatomical comparisons support the contention that the tunicate locus controls evo-

lution from the primitive foliaceous state which is typical of glumes in general to the highly specialized glumes of modern maize.

Vestigial glumes. The vestigial glume (*Vg*) mutant reported by Sprague (1939) is of particular morphological and agronomic significance. Originally this naked-flowered character was thought to be of no economic value because it was associated with pollen-blasting tassels. The discovery of modifying genes which usually permit abundant pollen production in *Vg* tassels, aroused interest in the utilization of the *Vg* character to improve the structure of the sweet corn (maize) ear (Galinat, 1951, 1953). It became apparent, however, that in order to insure pollen-production in inbred lines grown under various environmental conditions, it would be necessary to have tassels with glumes of normal length on *Vg* plants bearing glumeless ears. This desired *Vg* phenotype became a reality after a certain weak tunicate allele derived from the race "Chapalote" was combined with the *Vg* gene (Galinat, 1955, 1957). As a result, the agronomic development and testing of sweet corn hybrids with this new *Vg* phenotype is now in progress.

Vg glumes deviate from normal in the opposite direction from the deviation of tunicate glumes (Plates IX and X, figs. 2, 3, 4). In comparison to normal ones, *Vg* glumes are thicker, shorter and have increased lignification, while *Tu* glumes are thinner, longer and have reduced lignification (lacking the lignified region in the mesophyll). The progressive thickening of glumes in this series of three types (*Tu*; normal; *Vg*) appears to result largely from the development and proliferation of many small lignified cells in a region just under the abaxial epidermis. The smaller the cell in this region, the thicker the deposit of lignin in the secondary wall (Plate XI).

The accumulation of this lignin is known to be secondary, in that it occurs after the glumes have already attained optimum length and during the development of the caryopsis. If fertilization does not occur, then the glumes remain non-lignified and finally become papery. If only a portion of the ear is fertilized, then only those glumes which are either directly associated with fertilized ovaries or indirectly associated by being adjacent to spikelets with fertilized ovaries will become lignified. This immediate effect of fertilization in stimulating lignification of maternal tissue occurs also in teosinte and *Tripsacum*. Also the cupule of maize, as well as its counterpart in these close relatives, is included in the same metaxenial phenomenon.

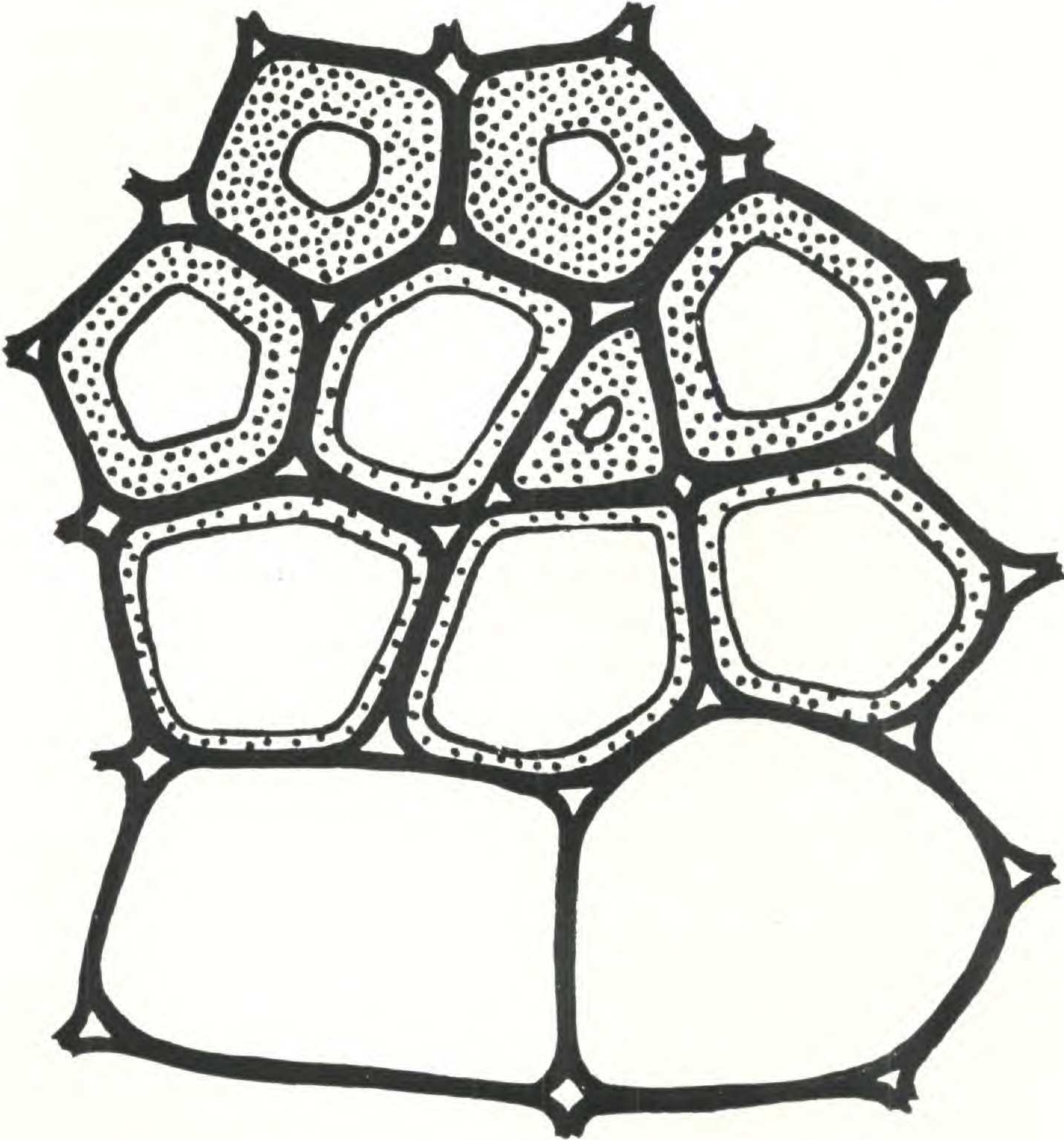
Certain features of the vestigial glume syndrome seem to be correlated with morphological homologies. The effect of the *Vg* gene in reducing glume size appears to be accomplished largely by suppression of the lateral wings (probably sheath-auricles), with the remaining portion representing a small, highly lignified sheath (compare figs. 3 and 4 in Plate IX). In addition to the lateral wings of the glumes, the *Vg* gene also causes, under certain conditions, a reduction of the lateral wings of the cupules and the ligules of the leaves; this last-named effect was noted first by Laughnan (1956). Glume wings and ligules have at least two features in common in that both of them are papyraceous in texture and are situated as erect prolongations of the sheath, or its homologue, adjacent to the actual or theoretical insertion-point of the blade. Furthermore, these structures appear to be homologous, as is suggested by the vegetative leaves of certain grasses (*Ammophila arundinacea*) with blades that are narrower than their sheaths. In such grasses, the lateral portions of the "ligule" elongate and develop as sheath-auricles with the same textural characters as the

EXPLANATION OF THE ILLUSTRATION

PLATE XI. A few contrasting cell types from a cross-sectional view of the outer pistillate glume of an ear with the vestigial glume character. Note that the smaller cells have a higher degree of lignification in the secondary walls (the stippled area). About 500 times natural size.

Drawn by WALTON C. GALINAT

PLATE XI



sheath, whereas the portion which is adjacent to the blade is reduced and papery. A similar phenomenon occurs in the lemmas of many grasses (*Avena* and *Bromus*) where the blade is modified into a narrow awn.

The effect of the *Vg* gene in reducing the size of the cupule wings is not necessarily comparable to its effects on either glume wings or ligules. Rather the small size of *Vg* spikelets may decrease their capacity to depress the adjacent rachis-internode during youth and thereby result in shallow cupules.

DISCUSSION

The features of *Tu* and *Vg* glumes differ from normal in opposite ways and in doing so they reflect glume-types involved in the early evolution of maize and *Tripsacum* in the American *Maydeae*. Teosinte is thought to have developed later from the hybridization of maize and *Tripsacum* (Mangelsdorf and Reeves, 1939). The foliaceous character of tunicate glumes is typical of the Andropogonaceous grasses (as in *Elyonurus tripsacoides*). Also it is a starting point from which the glumes of maize, *Tripsacum*, and possibly *Manisuris* could have evolved (Galinat, 1956). In the differentiation of *Tripsacum* and *Manisuris*, the glumes underwent reduction and lignification or sculpturing while the paired grain-bearing spikelets were reduced to singleness. Maize evolved in a separate direction which was controlled largely by mutation at the *Tu-tu* locus (Mangelsdorf, 1948). Nevertheless, a latent ability to evolve a *Tripsacoid*-type of outer glume might have been retained in modern maize and finally expressed as the *Vg* mutant. We conclude that in regard to this series (*Tu*; normal; *Vg*), the structure of tunicate glumes indicates that the *Tu-tu* locus may have been involved in previous evolution of the maize glume and that the mutation to the

vestigial glume condition demonstrates a potential in maize to evolve a *Tripsacoid*-type of glume.

The evolutionary changes resulting in shorter glumes during the domestication of maize could hardly have involved mutation at the *Vg-vg* locus. The *Vg* gene is a rare type of dominant mutation which seems to be of recent origin. Furthermore, it is not known to be part of a multiple-allelic series such as has been reported for the *Tu-tu* locus. There is also strong morphological evidence that the *Vg* gene has not been active in maize evolution. If such *Vg* activity had occurred, then it would be revealed by a characteristic reduction of the lateral wings of glumes. On the contrary, these wings are well-developed and papery in both modern and archaeological maize. In teosinte and *Tripsacum*, however, the glume wings are somewhat reduced and highly lignified.

In the series *Tu*; normal; *Vg*, decreases in glume-length are accompanied by increases in the thickness of the outer lignified zone. Since lignification of this outer zone of the glume occurs at the same time as kernel-development, there may be competition for the available energy. In the tunicate series of alleles (Mangelsdorf and Mangelsdorf, 1957) some of the energy conserved from shorter glumes is apparently diverted to increased grain production in spite of an increase in glume-lignification. Experiments are now in progress to determine the effect of the *Vg* gene on yield of mature grain.

Pn glumes exemplify a condition which does not fit into this pattern of variation and, notwithstanding the suggestion of Andrés (1950), could scarcely have exercised a protective role at any stage in the evolution of maize. The only possible evolutionary counterpart of papyrescent glumes in other grasses appears to be represented by a certain degenerate change found in several

varieties of *Sorghum* (Rangaswami and Panduranga, 1936). A papery condition which is apparently similar to that of papyrescent maize has been produced by two X-ray induced mutations in barley (Scholz, 1956).

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